Industries, Ltd., for providing intermediates and elemental analyses. This work was supported by Grant GM21747 from the National Institutes of Health. NMR data (250 MHz) was obtained at the Worcester Consortium NMR Facility at Clark University, supported by the National Science Foundation under Grant DMR 8108697.

Registry No. 1, 5759-80-8; 2, 60308-49-8; 5, 100763-38-0; 6, 100763-39-1; 7, 100763-40-4; 8, 100763-41-5; 9, 100763-82-4; 10, 91066-67-0; 11, 6944-08-7; 12, 100763-42-6; 13, 91333-15-2; 14, 100763-43-7; 15, 100763-44-8; 16, 100763-45-9; 17, 100763-46-0; 18, 100763-47-1; 19, 100763-48-2; 20, 100763-49-3; 21, 100763-50-6; 22, 100763-51-7; 23, 100763-52-8; 24, 100763-53-9; 25, 100763-54-0; 26, 100763-55-1; 27, 100763-56-2; 28, 100763-57-3; 29, 91769-84-5; 30, 100763-58-4; 31, 100763-59-5; 32, 24722-42-7; 33, 100763-60-8; 34, 100763-61-9; 35, 28484-80-2; 36, 100763-62-0; 37, 5770-18-3; 38, 100763-63-1; 39, 100763-64-2; 40, 100763-65-3; 41, 100763-66-4; 42, 7155-22-8; NaOPr, 6819-41-6; NaO-(c-C_6H_{11}), 22096-22-6; KoPh, 100-67-4; p-KOC_6H_4Me, 1192-96-7; m-KOC_6H_4Me, 36294-16-3; KOC_6H_3-3,4-diMe, 40590-38-3; p-KOC_6H_4Cl, 1121-74-0; p-KOC_6H_4NHAc, 35719-43-8; p-KOC_6H_4Et, 75121-14-1; p-KOC_6H_4Br, 3046-26-2; m-KOC_6H_4Et, 75121-13-0; p-KOC_6H_4Pr, 100763-67-5; p-$KOC_6H_4CH_2OH$, 100763-68-6; $PhCH_2NH_2$, 100-46-9; p-$NH_2C_6H_4Me$, 106-49-0; 2-amino-4-propoxy-6-chloropyrimidine, 100763-69-7; 2-amino-4-cyclohexyloxy-6-chloropyrimidine, 100763-70-0; 2-amino-4-phenoxy-6-chloropyrimidine, 100763-71-1; 2-amino-4-(p-tolyloxy)-6-chloropyrimidine, 100763-72-2; 2-amino-4-(m-tolyloxy)-6-chloropyrimidine, 100763-73-3; 2-amino-4-(3,4-xylyloxy)-6-chloropyrimidine, 100763-74-4; 2-amino-4-(p-chlorophenoxy)-6-chloropyrimidine, 100763-75-5; 2-amino-4-(p-acetamidophenoxy)-6-chloropyrimidine, 100763-76-6; 2-amino-4-(p-ethylphenoxy)-6-chloropyrimidine, 100763-77-7; 2-amino-4-(p-bromophenoxy)-6-chloropyrimidine, 100763-78-8; 2-amino-4-(m-ethylphenoxy)-6-chloropyrimidine, 100763-79-9; 2-amino-4-(p-propylphenoxy)-6-chloropyrimidine, 100763-80-2; 2-amino-4-(p-hydroxymethylphenoxy)-6-chloropyrimdine, 100763-81-3; 5-indanylamine, 24425-40-9; 2-amino-4,6-dichloropyrimidine, 56-05-3; 5-methyl-6-chlorouracil, 1627-28-7; 5-ethyl-6-chlorouracil, 20295-24-3; 6-chloro-s-triazine-2,4-dione, 69125-10-6; DNA polymerase, 9012-90-2.

Synthesis and Biological Activity of Several Amino Nucleoside–Platinum(II) Complexes

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Several platinum(II) complexes of 3′,5′-diamino-3′,5′-dideoxythymidine (compound **1**), 5′-amino-5′-deoxythymidine (compound **2**), and 3′-amino-3′-deoxythymidine (compound **3**) and the respective 2′-deoxyuridine amino nucleoside complexes, **4**–**6**, have been synthesized. Whereas compounds **1**, **2**, and **4**–**6** had no inhibitory effect on the replication of murine L1210 cells in cell culture, compound **3** [(3′-AdThd)$_2$PtCl$_2$] inhibited these cells with an ED_{50} of 0.8 μM. Incubation of L1210 cells with 10–20 μM compound **3** for 2 h produced less than 18% inhibition of RNA, DNA, or protein synthesis, which is of questionable significance. However a 16-h incubation resulted in an increased uptake of labeled thymidine into DNA (77%), labeled uridine into RNA (17%), and labeled amino acids into protein (100%). These unexpected results indicate that inhibition of macromolecules may not be involved in the inhibition of the replication of L1210 cells. The increased incorporation of labeled metabolites into macromolecules may be related to the increase in cell volume after a 2-h incubation of L1210 cells with compound **3** plus a marked increase after 2 h in the proportion of cells in their S phase. Compound **3** appears to delay the progression of cells through their cell cycle. A marked inhibitory effect on the transport of methionine or aminoisobutyric acid into L1210 cells was found with compound **3**, which was slightly greater than that produced with cisplatin. Compound **3** had a dose-dependent effect on the survival of mice bearing the L1210 ascites neoplasm, with a T/C × 100 of 175 at a dose of 320 mg/kg. Investigation of the kinetics of decomposition in aqueous systems demonstrated that the primary UV-absorbing decomposition product is 3′-amino-3′-deoxythymidine and that only a limited amount of the compound is formed (<8%). Although 3′-amino-3′-deoxythymidine could account for a part of the inhibition of the replication of L1210 cells in culture, it cannot account for the inhibition of amino acid transport by compound **3**, the platinum complex of 3′-amino-3′-deoxythymidine. Compound **3** has been shown to limit part of the amino acid uptake into L1210 cells in a similar manner to cisplatin.

Amino analogues of thymidine are of interest because of their biological activities. The 5′-amino analogue of thymidine was first synthesized by Horwitz et al.[4] and found to have good antiviral activity against the replication of herpes simplex virus both in vitro[5–8] and in vivo.[8] The corresponding 3′-amino analogue of thymidine was synthesized by both Miller and Fox[9] and Horwitz et al.[10] and found to have potent inhibitory activity against the replication of both murine Sarcoma-180 and L1210 cells in vitro[7,11] and in vivo.[12] The 3′,5′-diamino analogue of

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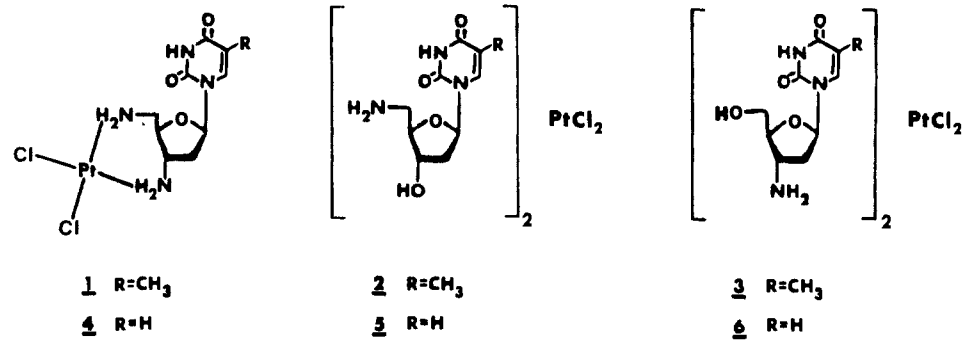


1 R=CH$_3$ 2 R=CH$_3$ 3 R=CH$_3$
4 R=H 5 R=H 6 R=H

Figure 1. Structures of several amino nucleoside–platinum(II) complexes.

thymidine was synthesized by Lin and Prusoff[7] and found to have neither antiviral nor antineoplastic activity.

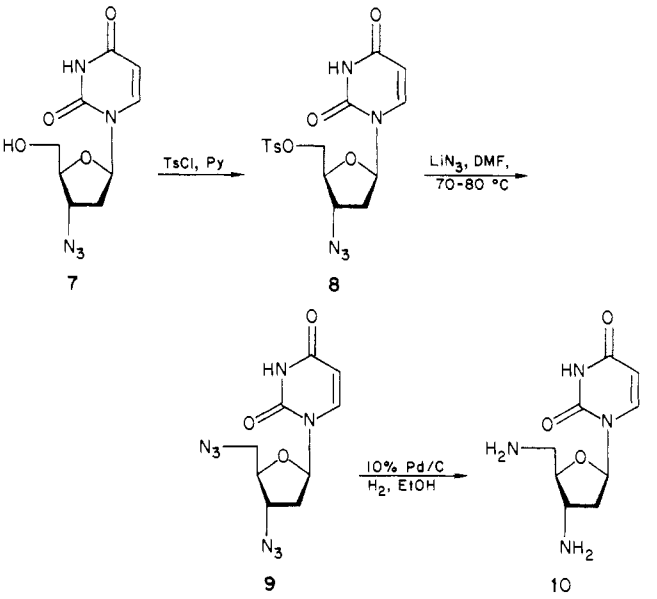
The corresponding nitrosourea analogues of both 3′-amino- and 5′-aminothymidine were synthesized by Lin et al.[13] and found to be potent inhibitors of L1210 cells in vitro[13] and murine L1210 and P388 leukemias in vivo.[14]

Several reviews have appeared over the past few years concerned with *cis*-dichlorodiammineplatinum(II) (cisplatin) and related compounds.[15-21] There are a number of improvements that would be desirable relative to the use of platinum complexes and these have been described by Burchenal et al.[22] These include (a) lack of cross-resistance to cisplatin; (b) a broader spectrum of activity; (c) increased clinical efficacy; (d) less severe side effects, less renal toxicity, and less emetic effects, since these toxicities limit the amount of this agent that patients can tolerate; (e) increased solubility for systemic use; (f) production of a synergistic effect when used in combination therapy; and (g) inhibition of the toxic, but not the therapeutic effects, of platinum compounds.

The need for platinum compounds with improved characteristics stimulated our synthesis of several amino nucleoside–platinum complexes. Our earlier finding,[14] that the use of thymidine as a carrier of the nitrosourea moiety (3′-CTNU) produced a compound with potent antineoplastic activity with unexpected but desirable pharmacological properties afforded the hope that a similar effect may be obtained with nucleoside–platinum complexes.

Of importance are the findings by Connors et al.[23] that the chloroplatinate complex of 1,2-diaminocyclohexane and of *o*-phenylenediamine have anticancer activity in mice against plasma cell neoplasms, and the finding by

Scheme I



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Burchenal et al.[24] that platinum-containing compounds that have their NH_3 ligands replaced by a 1,2-diaminocyclohexane moiety are active against animal tumors resistant to cisplatin.

Rose and Bradner[25] have recently reviewed several diaminocyclohexane–platinum(II) derivatives that have been synthesized and evaluated for biological activity.

We have synthesized six platinum complexes of the amino analogues of thymidine and 2′-deoxyuridine and found that one of these (compound **3**) inhibited the replication of L1210 cells in vitro and in vivo and the transport of methionine and aminobutyric acid into L1210 cells.

Chemistry. Various mono- and diamino nucleoside–platinum complexes (Figure 1, 1–6) have been synthesized by treatment of the respective mono- or diamino nucleosides with the appropriate molar ratio of potassium tetrachloroplatinate in water at 95–100 °C for 1 h or at room temperature for 24 h. The starting 5′-amino-5′-deoxythymidine, 5′-amino-2′,5′-dideoxyuridine, 3′-amino-3′-deoxythymidine (3′-AdThd), 3′-amino-2′,3′-dideoxyuridine, and 3′,5′-diamino-3′,5′-dideoxythymidine were fabricated by the previously reported methodology.[7,26] 3′,5′-Di-

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Table I. Stability of the Dinucleoside Platinum Complex (Compound 3) and Formation of 3′-AdThd upon Incubation at 37 °C in Selected Matrices

	compound 3,[a] %			formation of 3′-AdThd,[a] %		
time, h	H_2O	PBS[b]	5% Me_2SO/PBS	H_2O	PBS	5% Me_2SO/PBS
0.1	93.0	94.0	94.4	6.8	5.9	5.7
1.0	69.5	73.6	73.6	6.8	6.1	8.8
2.0	43.6	62.2	59.6	6.8	5.5	11.2
3.0	28.9	55.6	55.6	7.0	7.3	13.2
4.0	19.0	53.6	49.1	7.7	5.9	14.2
5.0	13.1	51.6	46.1	7.1	6.4	15.7
24.0	1.8	48.1	30.7	7.3	6.4	29.8

[a] Percent expressed relative to initial concentration of compound 3. [b] Phosphate-buffered saline, pH 7.4.

amino-2′,3′,5′-trideoxyuridine (10) was synthesized from 3′-azido-2′,3′-dideoxyuridine[27] (7) by tosylation, azide displacement, and catalytic hydrogenation as described in Scheme I.

Stability and Solubility of Compound 3 in Aqueous Solutions. The stability and dissolution properties of compound 3 in distilled water, PBS, and 5% Me_2SO/PBS were determined and compared. For measurement of rate of dissolution, 1.5 mg (0.002 mmol) of compound 3 was added to 20 mL of the solution of interest and sonicated for 4 min, and half of the solution was filtered. The resulting filtered and unfiltered solutions were incubated at 37 °C, and the absorbance of 268 nm was measured at 10-min intervals for 1–1.5 h. Absorbance of both the nonfiltered distilled water and PBS solutions of compound 3 increased through 1 h and then stabilized, indicating a very slow rate of dissolution. The filtered solutions remained constant in absorbance but at only about 60% of the theoretical absorbance based upon amount of compound weighed. The measured absorbance of the 5% Me_2SO/PBS solutions of compound 3, both filtered and nonfiltered, was equivalent to the calculated absorbance at the first scan, indicating immediate dissolution of compound 3 in this vehicle. These data should be considered when preparing solutions of compound 3 for biological and biochemical experiments.

There are marked differences in the stability of compound 3 upon incubation at 37 °C in these solutions (Table I). It appears that displacement of the 3′-AdThd ligands by H_2O, OH^-, or Cl^- found in the aqueous solutions does not occur to any significant extent. There appears to be no difference in the release of 3′-AdThd between the PBS and distilled water solutions, even though the free [Cl^-] is substantially lower in distilled water. However, addition of 5% Me_2SO as cosolvent with PBS produces a dramatic increase in 3′-AdThd with time. Me_2SO is known as a strong (S bonding) nucleophile toward Pt(II) and also exerts a very high trans effect.[28,29] Displacement of one of the chloride ligands by Me_2SO presumably labilizes the Pt–ligand bond trans to it and results in the release of 3′-AdThd through solvolysis. There is more than a four-fold increase in the release of 3′-AdThd at 24 h when 5% Me_2SO is present.

There are also dramatic differences in the rate of decomposition of compound 3 in each vehicle. The parent dichloro compound is most stable in PBS. The high (~150 mM) chloride ion concentration in PBS limits the degree of conversion (~52% at 24 h) to the mono- and diaquo and hydroxy species, which are interconvertible through multiple equilibria.[30] Distilled water allows virtually quantitative (1.8% remaining at 24 h) conversion of the parent dichloro compound to these other species. The presence of Me_2SO in PBS results in a loss of parent compound greater than that in PBS alone, due to the additional loss of parent compound through displacement of ligands by the nucleophilic Me_2SO ligand and additional labilization of other Pt–ligand bonds due to its trans effect.

Table II. Effect of the Dinucleoside–Platinum Complex (Compound 3), *cis*-Diamminedichloroplatinum(II) (Cisplatin), 3′-Amino-3′-deoxythymidine (3′-AdThd), and 3′-Amino-2′-3′-dideoxycytidine (3′-AdCyd) on Amino Acid Transport into L1210 Cells

		time in solution before addition to cells	% inhibition	
inhibitor	concn, μM		aminoisobutyric acid	methionine
3	10	<20 min	31	17
3	10	24 h	16	10
3′-AdThd	25	<20 min	<5	<5
3′-AdCyd	25	<20 min	<5	<5
cisplatin	10	<20 min	15–20	10
transplatin	10	<20 min	<5	<5

The implications of the solvent effects can be seen in the data on the effect of compound 3 and 3′-AdThd upon amino acid transport (Table II). If used immediately (<20 min), compound 3 inhibits transport of aminoisobutyric acid and methionine by 31% and 17%, respectively. However, if in solution for 24 h before used, the percent inhibition drops to 16% and 10%, corresponding to the 52% decomposition of compound 3 in PBS after 24 h. These data suggest that the inhibitory effect depends directly upon the concentration of intact drug present in the media. Furthermore, the inhibitory effect cannot be due to the release of 3′-AdThd, since under physiological conditions only about 7% is produced, or a concentration of 0.7 μM based on the initial 10 μM concentration of compound 3. Inhibition by 3′-AdThd alone at 25 μM is less than 5%. Thus the inhibition of amino acid transport cannot be due to compound 3 serving only as a prodrug for 3′-AdThd.

Biological Activity. The effect of these amino nucleoside–platinum complexes on the replication of murine L1210 cells in culture was investigated. Compounds 1, 2, and 4–6 did not inhibit the replication of L1210 cells; however, compound 3 proved to be a potent inhibitor with an ED_{50} of 0.8 μM.

The ED_{50} values were estimated from dose–response curves compiled from three independent experiments and represent the drug concentration required to inhibit the replication of L1210 neoplastic cells by 50%.

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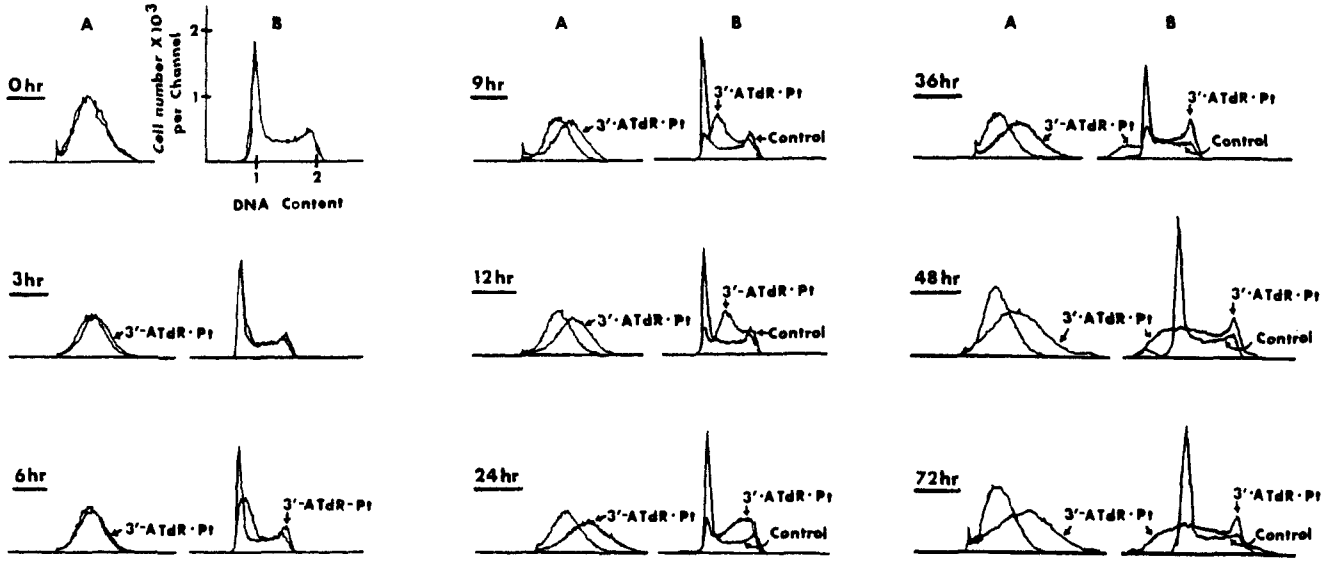

Figure 2. Changes in cell volume (A) and in cell-cycle distribution (B) of L1210 cells during continuous cell growth in presence of 15 μM dichlorobis(3'-amino-3'-deoxythymidine)platinum(II) complex.

Table III. Effect of the Dinucleoside–Platinum Complex (Compound **3**) on the Survival of Mice Bearing the L1210 Ascites Neoplasm

dosage, mg/kg	av days of survival	$T/C \times 100$
control	10.3	0
80	10.7	104
160	13.2	128
320	18.0	175

Table IV. Effect of the Dinucleoside–Platinum Complex (Compound **3**) on the Synthesis of RNA, DNA, and Protein by L1210 Cells

time of exposure of cells to **3**, h	observed effect	macromolecule		
		RNA, %	DNA, %	protein, %
2	inhibition	<18	<18	9
14	increase	77	17	100

The effect of compound **3** on relative cell volume and cell cycle was also determined. A twofold increase in cellular volume was observed by the third hour of incubation, which persisted for at least 72 h (Figure 2).

Between the third and sixth hour of incubation in the presence of compound **3** a marked decrease was observed in the proportion of cells in G_1 phase (Figure 2), as well as an accumulation of cells in the S phase, which slowly progressed to late S phase by 24 h. By 36 h there appeared to be a decrease in the number of S-phase cells approaching that of the control, an increase in the number of cells in the G_2-M phase, and a spreading prior to the G_1 peak, which may indicate degradation. Thus it appears that this compound has produced a slowing in the rate of progression through the cell cycle, as well as a degradation of the inhibited cell.

Compound **3** was selected for testing against mice bearing the L1210 leukemia. The results shown in Table III indicate a dose-dependent increase in survivors. However, compound **3** is considerably less potent than cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, based on the report of Macquet and Butow.[31] These investigators reported a $T/C \times 100$ of 205 when a *single* injection of cisplatin was administered at a dose of 8 mg/kg, whereas it required the administration of compound **3** at a dose of 320 mg/kg twice daily for 3 consecutive days in order to achieve a $T/C \times 100$ of only 175. Thus cisplatin is considerably more potent than compound **3** as an anticancer agent against the L1210 neoplasm in mice.

Biochemical Evaluation. 1. **Macromolecular Synthesis.** Treatment of L1210 cells for 2 h with compound **3** in five experiments resulted in an average of less than 18% inhibition of either RNA or DNA synthesis and in three experiments a 9% decrease in protein synthesis (Table IV). These inhibitions are not considered to be significant relative to the inhibition of cellular replication.

Incubation of L1210 cells with compound **3** for 16 h produced an increase in the uptake of labeled thymidine (77%) into DNA, of labeled uridine into RNA (17%), and of labeled amino acid into protein (100%) (Table IV). These unusual findings may be related to the marked increase in volume of L1210 cells when exposed to compound **3** for 16 h but not for 2 h, as well as the marked increase in the proportion of cells in S phase at 16 h (Figure 2).

2. Amino Acid Transport. The effect of compound **3**, cisplatin, 3'-amino-3'-deoxythymidine, and 3'-amino-3'-deoxycytidine on initial amino acid transport in L1210 cells was determined, and the data are presented in Table II. The experimental procedure has been described previously by Scanlon et al.[32]

Under conditions in which cisplatin produced a 10% inhibition of methionine transport or a 15–20% inhibition of aminoisobutyric acid transport, the nucleoside–platinum complex (compound **3**) produced a 17% and 31% inhibition, respectively. If the time of storage of compound **3** in aqueous solution was extended from 20 min to 24 h, then a significant decrease in inhibition was observed, presumably because of a decrease in the inhibitory species.

Because 3'-amino-3'-deoxythymidine (3'-AdThd) is formed to a limited amount (<8%) when compound **3** is dissolved in an aqueous solution, and therefore could be involved in the inhibitory effect on amino acid transport, the potential inhibitory effect of 3'-AdThd was evaluated. Although no inhibition was observed with 3'-AdThd or by the related deoxycytidine analogue (3'-AdCyd), it is conceivable that either compound **3** per se or another degra-

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dation product(s) is responsible for the inhibitory effect. This is under investigation. Thus compound **3** inhibits transport of amino acid to a greater extent than does cisplatin.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 60 MHz on a Varian T-60 spectrometer or at 270 MHz on a Brucker 270 HX spectrometer with Me_4Si as the internal reference. The UV spectra were recorded on a Beckman-25 spectrophotometer. IR spectra were taken on the Perkin-Elmer 21 spectrophotometer. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

General Procedure for the Preparation of Amino Nucleoside–Platinum(II) Complexes 1–6. A solution of the amino nucleoside (1.0 mmol) in 15 mL of water was added dropwise to a solution of potassium tetrachloroplatinate (2.0 mmol) in 15 mL of water. The reaction mixture was stirred at 95–100 °C for 1 h or at room temperature for 24 h, during which time a dark yellow to yellow solid precipitate was formed. The solid material was pulverized and collected by filtration, washed several times with water and then with appropriate amount of ethanol and ether, and dried to yield the desired final product.

In case of the fabrication of the diamino nucleoside–platinum(II) complexes **1** and **4**, a molar ratio of 1:1 instead of 2:1 of the respective diamino nucleoside and potassium tetrachloroplatinate was employed.

***cis*-Dichloro(3′,5′-diamino-3′,5′-dideoxythymidine)platinum(II) Complex (1)**: yield, 84%; mp 274–276 °C dec; UV (0.01 N HCl) λ_{max} 266 nm (ϵ 10370); UV (0.01 N HCl) λ_{min} 241 nm; UV (0.01 N NaOH) λ_{max} 267 nm (ϵ 8310); UV (0.01 N NaOH) λ_{min} 248 nm; NMR (Me_2SO-d_6) δ 1.80 (s, 3 H, 5-CH_3), 2.20–2.45 (m, 2 H, 2′-H), 3.40–360 (m, 2 H, 5′-H), 6.08 (m, 1 H, 1′-H), 7.45 (s, 1 H, 6-H), 11.3 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{10}H_{16}Cl_2N_4O_3Pt$) C, H, Cl, N.

Dichlorobis(5′-amino-5′-deoxythymidine)platinum(II) Complex (2): yield, 47%; mp 214–215 °C dec; UV (0.01 N HCl) λ_{max} 268 nm (ϵ 17000); UV (0.01 N HCl) λ_{min} 243 nm; UV (0.01 N NaOH) λ_{max} 268 nm (ϵ 14870); UV (0.01 N NaOH) λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 1.80 (s, 6 H, 5-CH_3), 2.00–2.40 (m, 4 H, 2′-H), 3.30–3.45 (m, 4 H, 5′-H), 3.85–4.10 (m, 2 H, 4′-H), 4.12–4.28 (m, 2 H, 3′-H), 5.20–5.60 (br s, 2 H, 3′-OH, D_2O exchangeable), 6.20 (m, 2 H, 1′-H), 7.60 (s, 2 H, 6-H). Anal. ($C_{20}H_{30}Cl_2N_6O_8Pt{\cdot}4H_2O$) C, H, N, Cl.

Dichlorobis(3′-amino-3′-deoxythymidine)platinum(II) Complex (3): yield, 71%; mp 204–206 °C dec; UV (0.01 N HCl) λ_{max} 268 nm (ϵ 17990); UV (0.01 N HCl) λ_{min} 241 nm; UV (0.01 N NaOH) λ_{max} 268 nm (ϵ 14200); UV (0.01 N NaOH) λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 1.75 (s, 6 H, 5-CH_3), 2.02–2.32 (m, 4 H, 2′-H), 3.64–3.82 (m, 4 H, 5′-H), 4.05–4.18 (m, 2 H, 4′-H), 4.90–5.00 (m, 2 H, 3′-H), 5.02–5.19 (m, 2 H, 5′-OH, D_2O exchangeable), 6.22 (m, 2 H, 1′-H), 7.70 (s, 2 H, 6-H), 11.2 (br s, 2 H, 3-NH, D_2O exchangeable). Anal. ($C_{20}H_{30}Cl_2N_6O_8Pt$) C, H, Cl, N.

***cis*-Dichloro(3′,5′-diamino-2′,3′,5′-trideoxyuridine)platinum(II) Complex (4)**: yield, 90%; mp 271 °C dec; UV (EtOH) λ_{max} 261 nm (ϵ 10310); UV (EtOH) λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 2.20–2.40 (m, 2 H, 2′-H), 3.30–3.50 (m, 2 H, 5′-H), 3.70–3.90 (m, 1 H, 4′-H), 4.20–4.60 (m, 1 H, 3′-H), 5.70 (d, 1 H, 5-H), 6.10 (m, 1 H, 1′-H), 7.62 (d, 1 H, 6-H), 11.3 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{14}Cl_2N_4O_3Pt$) C, H, Cl, N.

Dichlorobis(5′-amino-2′,5′-dideoxyuridine)platinum(II) Complex (5): yield, 42%; mp 210–211 °C dec; UV (H_2O) λ_{max} 261 nm (ϵ 17750); UV (H_2O) λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 2.10–2.41 (m, 4 H, 2′-H), 3.40–3.60 (m, 4 H, 5′-H), 3.82–4.46 (m, 4 H, 4′- and 3′-H), 5.70 (d, 2 H, 5-H), 6.18 (m, 2 H, 1′-H), 7.68 (d, 2 H, 6-H). Anal. ($C_{18}H_{26}Cl_2N_6O_8Pt{\cdot}2H_2O$) C, H, Cl, N.

Dichlorobis(3′-amino-2′,3′-dideoxyuridine)platinum(II) Complex (6): yield, 63%; mp 207–208 °C dec; UV (H_2O) λ_{max} 263 nm (ϵ 16490); UV (H_2O) λ_{min} 243 nm; NMR (Me_2SO-d_6) δ 2.10–2.40 (m, 4 H, 2′-H), 3.50–3.70 (m, 4 H, 5′-H), 3.72–3.95 (m, 2 H, 4′-H), 3.96–4.18 (m, 2 H, 3′-H), 3.68 (br d, 2 H, 5′-H), 5.00 (br s, 2 H, 5′-OH, D_2O exchangeable), 6.20 (d, 2 H, 5-H), 7.80 (d, 2 H, 6-H). Anal. ($C_{18}H_{26}Cl_2N_6O_8Pt{\cdot}2H_2O$) C, H, Cl, N.

3′-Azido-5′-*O*-(*p*-tolylsulfonyl)-2′,3′-dideoxyuridine (8). *p*-Toluenesulfonyl chloride (4.66 g, 24.5 mmol) was added to an ice-cooled solution of 3′-azido-2′,3′-dideoxyuridine[27] (5.17 g, 20.4 mmol) in 30 mL of dry pyridine. The reaction mixture was stirred at 4 °C for 48 h, and the solvent was removed in vacuo. The resulting glassy residue was dissolved in 25 mL of EtOH and was then added dropwise into 600 mL of vigorously stirred ice–water. The white-gray precipitate was collected by filtration and dried to yield 6.75 g (82%) of crude product, which was then purified by recrystallization from EtOH to give white crystals: mp 90–92 °C; UV (EtOH) λ_{max} 261 nm (ϵ 10190); UV (EtOH) λ_{min} 241 nm; NMR (Me_2SO-d_6) δ 2.40–2.60 (m, 2 H, 2′-H), 3.81–4.08 (m, 1 H, 4′-H), 4.25–4.40 (m, 3 H, 3′- and 5′-H), 5.42–5.60 (br d, 1 H, 5-H), 6.00 (t, 1′-H), 7.40 (d, 3 H, 6-H; and H_A, aromatic ring), 7.71 (d, 2 H, H_B, aromatic ring), 11.2 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{16}H_{17}N_5O_6S$) C, H, N, S.

3′,5′-Diazido-2′,3′,5′-trideoxyuridine (9). A mixture of **8** (2.3 g, 5.6 mmol) and lithium azide (0.5 g, 10.2 mmol) in 12 mL of DMF was stirred at 70–80 °C for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved in 5 mL of warm EtOH, and the solution was then added dropwise into a mixture of EtOH–H_2O (60 mL, 2:1, v/v). The resulting solution was kept in a refrigerator overnight. The white crystals formed were collected by filtration and dried to afford 1.24 g (80%) of product: mp 67–68.5 °C; IR (KBr) 4.75 μm (azido); UV (EtOH) λ_{max} 260 nm (ϵ 9830); UV (EtOH) λ_{min} 232 nm; NMR (Me_2SO-d_6) δ 2.25–2.66 (m, 2 H, 2′-H), 3.48–3.76 (m, 2 H, 5′-H), 3.80–4.18 (m, 1 H, 4′-H), 4.20–4.61 (m, 1 H, 3′-H), 5.70 (d, 1 H, 5-H), 6.16 (t, 1 H, 1′-H), 7.70 (d, 1 H, 6-H), 11.3 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{10}N_8O_3$) C, H, N.

3′,5′-Diamino-2′,3′,5′-trideoxyuridine (10). A solution of compound **9** (0.46 g, 1.60 mmol) in 15 mL of EtOH was hydrogenated under 50 psi of hydrogen for 48 h, in the presence of 0.1 g of 10% Pd/C. At the end of the reduction, the mixture was heated to 60–70 °C and then filtered. The filtrate was evaporated in vacuo to afford 0.35 g (97%) of white solid, which was used immediately for the preparation of the corresponding platinum complex **4** without further purification: NMR (Me_2SO-d_6) δ 1.86–2.28 (m, 2 H, 2′-H), 2.67–2.91 (m, 2 H, 5′-H), 3.30–3.74 (m, 6 H, 3′- and 4′-H; and 3′- and 5′-NH_2, D_2O exchangeable), 5.60 (d, 1 H, 5-H), 6.10 (t, 1 H, 1′-H), 7.75 (d, 1 H, 6-H).

Determination of Stability of Compound 3 in Selected Matrices. Compound **3** (1.5 mg, 0.002 mmol) was added to 20 mL of distilled water, PBS, or 5% Me_2SO/PBS, sonicated and stirred for 4 min, and filtered through a LC13 Acrodisc (Gelman Sciences, Inc., Ann Arbor, MI). The solution was incubated at 37 °C, and 100-μL aliquots for analysis by reversed-phase liquid chromatography were withdrawn at 5 min, 1, 2, 3, 4, 5, and 24 h. A Whatman Partisil ODS-2 (25 × 0.46 cm, 10 μm) column was used. Mobile phase A was 0.01 M KH_2PO_4 (pH 3.0)–5% methanol and mobile phase B was 0.01 M KH_2PO_4 (pH 3.0)–45% methanol. The linear gradient used was 0–100% B in 30 min at a flow rate of 1.75 mL/min at ambient temperature. The chromatographic system and these conditions have been described previously.[33] Calibration curves for compound **3** and for 3′-AdThd were constructed by multiple injections of standard solutions of each at three concentrations in the range of interest. Concentrations of compound **3** and 3′-AdThd found at each time point are expressed as percent of the initial concentration of compound **3**, determined by UV scan of an aliquot immediately following the filtration step.

Biological and Biochemical Procedures. 1. In Vitro. Mouse L1210 leukemia cells were maintained as suspension cultures in Fischer's medium supplemented with 10% horse serum at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Under these conditions, the generation time for L1210 cells is ca. 12 h. Each compound at several concentrations was added to L1210 cells (2×10^4 cells/mL), which were in their exponential phase of growth. The increase in cell number of drug-free culture (control), as well as that of the cultures supplemented with the test compounds, was determined after 24, 48, and 72 h of growth.

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2. In Vivo. Transplantation of L1210 ascites cells was carried out by withdrawing peritoneal fluid from donor CDF_1 mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600*g*), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter, and the cell population was adjusted to 10^6 cells/mL. One-tenth milliliter of the resulting cell suspension (containing ca. 10^5 cells) was injected intraperitoneally into each animal. Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, twice daily for 3 consecutive days. The test compounds were injected as a solution of isotonic saline. All drugs were administered intraperitoneally in a volume of 0.25 mL. For any one experiment, animals were distributed into groups of six mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water "ad libitum". Controls, given injections of a comparable volume of vehicle, were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of ascitic neoplasms to those agents were based on the prolongation of survival time afforded by the drug treatments.

3. Determination of the Effect of Compound 3 on Relative Cell Volume and Cell Cycle of L1210 Cell. Three milligrams of compound **3** was suspended in 10 mL of H_2O, stirred for 10 min, and filtered, and the concentration was determined by measurement of absorbancy of 268 nm ($A = 1.7 \times 10^4$). L1210 cells (1×10^5 cells/mL) in Fischer's medium with and without compound **3** (15 μM) in a final volume of 50 mL were incubated for 0–72 h at 37 °C.

At appropriate times a volume containing 1×10^6 cells was removed and fixed for staining as follows: the cell suspension is centrifuged at 160*g* for 5 min, the supernate is decanted, and the pellet is suspended in 2 mL of cold PBS. Two milliliters of cold 95% EtOH is added to the cell suspension and subjected to vortex mixing, this is followed by addition of three 2-mL portions of cold EtOH with mixing after each addition. After at least 20 min, the cells are stained with Mithramycin as described.[34,35] At varying increments of time, the fixed and stained cells were subjected to analysis with a Becton-Dickinson Fluorescence Activated Cell Sorter IV. The wavelength used for excitation was 457 nm; laser power, 50 mW; emitted light collected using 515, 520 LP filter; number of L1210 cells collected per sample 30 000; Scale-forward scatter, 256 channels, linear; and fluorescence 1, 256 channels, log.

4. Determination of the Effect of Compound 3 on the Macromolecular Synthesis of L1210 Cells. Cell Culture. Suspension cultures of murine leukemia L1210 cells were grown in Fisher's medium at 37 °C supplemented with 10% horse serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) (Grand Island Biological Co., Grand Island, NY) in a 5% carbon dioxide incubator. Asynchronous, exponentially growing cells were used in all experiments. A Model ZBI Coulter counter was used for counting cells.

Chemicals. [*methyl*-^{3}H]Thymidine (50 Ci/mmol) and [5-^{3}H]uridine (20 Ci/mmol) were from Moravek Biochemicals (Brea, CA). [^{14}C]Protein hydrolysate (57 mCi/mmol and [^{35}S]methionine (1035 Ci/mmol) were from Amersham Corp. Compound **3** was suspended in H_2O, stirred for 10 min, and filtered, and the concentration was determined by measurement of absorbancy at 268 nm ($A = 1.7 \times 10^4$).

Reaction Mixture. The L1210 cells were centrifuged for 5 min at 160*g* and resuspended in fresh Fisher's medium at a concentration of 2×10^5 cells/mL in a total volume of 45 mL. Compound **3**, as prepared above, was diluted 10- to 20-fold and added to the cell suspension for a final concentration of 10–20 μM in an incubation volume of 50 mL. Two- or 5-mL volumes were removed after 2 or 16 h of incubation with compound **3**, and the appropriate radioactive metabolite was added to produce the following concentration: [^{3}H]thymidine (2 μCi/mL); [^{3}H]uridine (2 μCi/mL); [^{14}C]protein hydrolysate (1.25 μCi/mL), and [^{35}S]-methionine (5 μCi/mL).

After 0.5 and 2.0 h of incubation with the labeled metabolite, the cells were harvested by centrifugation, and the pellet was washed with cold PBS two times and then resuspended in 2–5% EDTA. Twenty-five microliters was spotted on Whatman No. 1 filter paper disk (2.4-cm diameter), washed with 5% TCA twice and once with 95% ethanol, air-dried, and counted for their content of radioactivity.

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